II. RESPONSE TO OFFICE ACTION

A. Status of the Claims

Claims 35, 69, 74-77, 91-111, and 114-134 were pending in the case at the time of the Office Action. Claims 69, 74, 98, and 103 have been amended as set forth herein. Claims 1-68, 70-73, 78-90, 105-107, 112-113, and 131 have been canceled without prejudice or disclaimer. No new claims have been added. Support for the amendments of the claims can be found generally throughout the specification, such as in the claims as originally filed. Additional support for particular amendments is discussed in greater detail below. Thus, claims 69, 74-77, 91-104, 108-111, 114-130, and 132-134 are currently under consideration.

B. The Written Description Rejections Under 35 U.S.C. §112, First Paragraph, Are Overcome

Claims 69, 74, 98, 103, 124-126, 128, 129, and 134 are rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement. The claims are said to contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. The Examiner argues that the original disclosure does not support claims directed to the claimed ranges of molar ratios. Applicants respectfully traverse.

The Federal Circuit has stated that the test for the written description requirement is "whether the application relied upon 'reasonably conveys to the artisan that the inventor had possession at the time of the later claimed subject matter.'" *In re Daniels*, 144 F.3d 1452, 1456, 46 USPQ2d 1788, 1790 (Fed. Cir. 1998). See also *Markman v. Westview Instruments, Inc.* 52 F.3d 967, 34 USPQ 2d 1321 (Fed. Cir. 1995) (*en banc*) ("Claims must be read in view of the

specification, of which they are a part."). In rejecting a claim under the written description requirement of 35 U.S.C. §112, first paragraph, the Examiner has the initial burden of presenting evidence or reasons why a person skilled in the art would not recognize in an applicant's disclosure a description of the invention defined in the claims. *In re Wertheim*, 541 F.2d 257, 262, 191 USPQ 90, 96 (CCPA 1976). Accordingly, the Examiner is required: (1) to set forth the claim limitation not described; and (2) to provide reasons why a person skilled in the art would not have recognized the description of the limitation in view of the disclosure of the application as filed. *Interim Guidelines for the Examination of Patent Applications Under 35 USC 112, Paragraph 1*; Chisum on Patents, vol. 3, §7.04[1][c].

The rejected claims recite specific range limitations. For example, claim 69 recites the limitation "wherein the molar ratio of basic reagent:dye in the composition is 1:1 to 25:1." Support for the range limitations recited in the claims can be found, for example, in the paragraph bridging pages 4-5 of the instant specification. The specification recites the specific upper end and lower end ratio of the ranges. Further, the specification notes that included in the claimed ratios of dye and basic reagent are "all the intermediate ranges as well" and all ranges in between these values." Specification, page 4, line 31 and page 5, line 2.

The Examiner appears to find objectionable that the specification does not include a specific, word for word recitation of the claimed ranges. However, in setting forth such a rejection, she misconstrues the written description requirement. The Federal Circuit has stated that "[t]he written description requirement does not require the applicant 'to describe exactly the subject matter claimed, [instead] the description must clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed.'" *Union Oil Co. of California v. Atlantic Richfield Co.*, 208 F.3d 989, 997, 54 USPQ 2d 1227, 1232 (Fed. Cir. 2000). The

Federal Circuit has also noted that "[if] a person or ordinary skill in the art would have understood the inventor to have been in possession of the claimed invention at the time of filing, even if every nuance of the claims is not explicitly described in the specification, then the adequate written description requirement is met." *In re Alton*, 76 F.3d 1168, 1175, 37 USPQ2d 1578, 1584 (Fed. Cir. 1996). See also *Purdue Pharma L.P. v. Faulding Pharmaceutical Co.*, 230 F.3d 1320, 1323, 56 USPQ2d 1481, 1483 (Fed. Cir. 2000) (The disclosure as originally filed need not provide "*in haec verba* support for the claimed subject matter at issue," rather, the disclosure should convey to one skilled in the art that the inventor was in possession of the invention at the time of filing).

The Examiner also argues that there is no written description because the working examples do not support the claimed ranges. Applicants note that the molar ratio of basic reagent:dye in the gendine solution of Example 1 is about 1:1. Nevertheless, Applicants note that they are not required to set forth working examples in the specification in order to meet the written description requirement. Rather, the standard is as set forth above. The Examiner also appears to argue that Applicants' previously submitted supplemental data does not support written description of the claimed invention. See Office Action, page 2, section 2. However, Applicants' supplemental data was previously submitted to support nonobviousness of the claimed invention, and not necessarily to support written description.

It is respectfully submitted that a person of ordinary skill in the art, when presented with the instant specification, would have understood that Applicants were in possession of the claimed ranges of ratios of dye and basic reagent. No evidence to the contrary has been set forth by the Examiner.

Regarding the Examiner's concern related to the recitation of D&C Yellow No. 1 in claims 69 and 74, Applicants have rendered this issue moot by canceling this dye from the claims. Applicants specifically reserve the right to prosecute the subject matter canceled from these claims in a divisional or continuation application.

For each of the foregoing reasons, the written description rejection of claims 69, 74, 98, 103, 124-126, 128, 129, and 134 under 35 U.S.C. §112, first paragraph, should be withdrawn.

C. The Rejections Under 35 U.S.C. §102 Are Overcome

1. The Rejections Based on Cid Are Moot

Claims 103, 104, 108, 130, and 134 are rejected under 35 U.S.C. §102(b) as being anticipated by Cid (ES 2061407). Applicants traverse.

Without conceding that the claims as originally written were anticipated by Cid, Applicants note that claim 103 has been amended to recite the limitations of claim 131, a claim which was not included in this rejection and thus considered to be novel over Cid. The remaining claims at issue in this rejection depend from claim 103, and would also thus be novel over Cid. Applicants specifically reserve the right to pursue the subject matter of the claims as originally written in a divisional or continuation application. In view of the foregoing, the rejection of claims 103, 104, 108, 130, and 134 is moot.

2. Rejection of Claims 103, 104, and 131-134 Based on Perelin

Claims 103, 104, and 131-134 are rejected under 35 U.S.C. §102(e) as being anticipated by Perelin (U.S. 2002/0009693). The Examiner argues that Perelin discloses the use of gentian violet in a chlorhexidine-containing disinfecting composition for application to an organic surface. Applicants traverse.

Without conceding that the claims as originally written would have been anticipated by Perelin, Applicants note that independent claim 103 has been amended to recite the limitations of claim 131, and thus now recites that the surface is a mucosal surface. Further, each of the remaining claims at issue in this rejection depend from claim 103, and thus includes the limitations of claim 103.

Applicants have reviewed Perelin, and do not find where it discloses application of a composition containing chlorhexidine and gentian violet to a mucosal surface. Rather, Perelin is directed to dental restoration solutions for root and dentinal tubule treatment. See, *e.g.*, title, abstract, and paragraph [0005]. Attached as Exhibit A are pages from Stedman's Medical Dictionary. Page 218 recites "See dentinal canal" under the definition of "dentinal tubule." Page 218 recites the following definition of "dentinal canal": "Any of the minute, wavy, branching tubes in the dentin that contain dentinal fibers and extend radially from the pulp to the dentoenamel junction. Also called *dentinal tubule*." On page 729, the definition of "root" includes the following: "The embedded part of an organ or structure, such as a hair, tooth, or nerve, serving as a base or support." Thus, root and dentinal tubule in the context of Perelin concern parts of a tooth, and not a mucosal surface. Further, Perelin does not appear to make any reference to any method of disinfecting or sterilizing a mucosal surface.

Therefore, because Perelin fails to teach or suggest each limitation of the claimed invention, it fails to anticipate the instant claims. Applicants therefore respectfully request that the Examiner withdraw the rejection of claims 103, 104, and 131-134 under 35 U.S.C. §102(e) based on Perelin.

3. Rejection of Claims 109-111 Based on Perelin

Claims 109-111 are rejected under 35 U.S.C. §102(b) as being anticipated by Perelin (U.S. 2002/0009693). The Examiner argues that Perelin discloses a method of disinfecting a

wound (exposed dental root) by applying a composition containing gentian violet and chlorhexidine thereto. Applicants traverse.

Without conceding that the claims as originally written would have been anticipated by Perelin, Applicants note that independent claim 109 has been amended to recite that the wound is a "skin wound." Support for the amendment of claim 109 can be found generally throughout the specification, such as on page 6, lines 13-16 and page 7, lines 31-page 8, line 2. Applicants find no disclosure in Perelin pertaining to a skin wound, and thus Perelin fails to anticipate claim 109, and dependent claims 110-111.

Therefore, Applicants respectfully request that the rejection of claims 109-111 under 35 U.S.C. §102(b) as being anticipated by Perelin be withdrawn.

D. The Rejections Under 35 U.S.C. §103(a) Are Overcome

1. Rejection Based on Fenn *et al.* in View of Dodd *et al.* is Moot

Claim 35 is rejected under 35 under 35 U.S.C. §103(a) as being unpatentable over Fenn *et al.* (GB 2007096) in view of Dodd *et al.* (U.S. Patent 6,344,218). Applicants respectfully traverse.

Without conceding that claim 35 is unpatentable over Fenn *et al.* in view of Dodd *et al.*, Applicants have canceled this claim without prejudice or disclaimer. Applicants reserve the right to prosecute this claim in a continuation or divisional application. In view of the foregoing, the rejection of claim 35 under 35 under 35 U.S.C. §103(a) as being unpatentable over Fenn *et al.* in view of Dodd *et al.* is moot.

2. Rejections Based on Luthra in View of Perelin Are Overcome

Claims 69, 74-77, 91-96, 99, 100, 114-119, 121, 122, and 124-129 are rejected under 35 U.S.C. §103(a) as being unpatentable over Luthra *et al.* (WO 00/65915; "Luthra") in view of

Pelerin (as above). The Examiner argues that Luthra teaches a method of disinfecting a medical device by applying a composition containing chlorhexidine, and that one of ordinary skill in the art would be motivated to include a dye in the composition because Perelin is said to disclose use of gentian violet in a chlorhexidine-containing disinfecting composition to indicate to the user where the composition has been applied. Applicants respectfully traverse.

a. The Examiner has Failed to Set Forth a *Prima facie* Case of Obviousness

It is well settled that the Examiner bears the initial burden of factually supporting any *prima facie* case of obviousness. *MPEP* § 2142. In making a determination as to whether a *prima facie* case of obviousness exists, the examiner should: (A) determine the "scope and content of the prior art;" (B) ascertain the "differences between the prior art and the claims at issue;" (C) determine "the level of ordinary skill in the pertinent art;" and (D) evaluate evidence of secondary considerations. *Graham v. John Deere*, 383 U.S. 1, 17, (1966) ; *see also KSR Int'l Co. v. Teleflex Inc.*, 127 S.Ct. 1727, 1734 (2007) ("While the sequence of these questions might be reordered in any particular case, the [Graham] factors continue to define the inquiry that controls.").

The Action fails to establish that a person of ordinary skill in the art would have had an "apparent reason" to combine the teachings of Luthra and Perelin in the fashion claimed. *KSR*, 127 S.Ct. at 1740-41. Regarding Perelin, the Examiner incorrectly argues that Luthra teaches a method of disinfecting a medical device by applying a composition containing chlorhexidine to the device. Luthra does not teach applying any such composition to a surface of a medical device, but rather concerns itself with devices composed of polymers, wherein the polymer includes a biguanide moiety. See, *e.g.*, abstract and page 6, lines 4-20. Thus, no application of a chlorhexidine composition to the surface of a medical device (as set forth in claim 69 and

dependent claims) or any application of chlorhexidine for disinfecting or sterilizing a fluid (claim 74 and dependent claims) appears to be disclosed in Luthra.

Further, as admitted by the Examiner, Luthra does not disclose any dye-containing composition. To attempt to remedy this deficiency of Luthra, the Examiner cites Perelin. Perelin, as discussed above, concerns itself with application of particular dental restoration solutions for the identification of parts of a tooth including the root and dentinal tubule of the tooth without the mention of a method for the sterilization of a mucosal surface. *See, e.g.*, abstract and paragraph [0004]. Per paragraph [0012] of Perelin, "[a] dye is optionally present to identify a tooth region contacted with the inventive solution." One of ordinary skill in the art would not be motivated to combine the teachings of Perelin with Luthra because Luthra, as discussed above, does not concern application of a chlorhexidine-containing solution to a medical device (as required by claim 69 and dependent claims). Further, nothing in either reference provides any teaching or suggestion for a method of sterilizing or disinfecting a fluid.

Thus, for each of the foregoing reasons, the Examiner has failed to establish a *prima facie* case of obviousness of claims 69, 74-77, 91-96, 99, 100, 114-119, 121, 122, and 124-129 under 35 U.S.C. §103(a) based on Luthra in view of Perelin.

b. Applicants' Evidence of Surprising and Unexpected Results is Sufficient to Rebut an Obviousness Challenge

Even if the Examiner argues that a *prima facie* case of obviousness has been made, which Applicants as discussed above assert is not the case, Applicants would still overcome any obviousness rejection because Applicants' methods are surprisingly and unexpectedly superior compared to methods using either dye alone or basic reagent alone.

As Applicants have previously set forth in their response to the Office Action dated August 21, 2007, the Example section of the specification provides strong evidence of synergy of

gentian violet (GV) and chlorhexidine (CHX) as an antiseptic/disinfectant. Table 2 and Table 3 on page 20 of the referenced patent application show zones of inhibition (ZOI) produced by coated endotracheal PVC tubes (using DCM or MeOh). As set forth in the application on page 20, lines 16-19, "endotracheal PVC tubes impregnated with Gendine (GN) are far more effective against all organisms when compared with those impregnated with CHX, and more effective than PVC tubes impregnated with GV against *Pseudomonas aeruginosa.*"

Table 4 on page 21 of the referenced application shows ZOI produced by coated silicone catheters. Page 21, lines 10-12 states that "data in Table 4 shows how silicone catheters impregnated with GN are more effective in inhibiting *MRSA, PS* and *C. parapsilosis* than catheters impregnated with either GV or CHX."

Table 5, on page 21 of the present application, shows ZOI produced by coated polyurethane catheters (PU). Page 21, lines 24-27 states that "PU catheters impregnated with GN are more effective than PU catheter impregnated with GV in inhibiting *Pseudomonas aeruginosa,* and more effective than PU catheters impregnated with CHX against all three organisms, *MRSA, PS* and *C. parapsilosis.*"

Table 6, on page 22 of the present application, shows ZOI produced by coated silk sutures. Page 21, lines 10-12 provides that "silk sutures coated or impregnated with GN are significantly more effective in inhibiting MRSA, PS and *C. parapsilosis* than sutures coated with either GV or CHX."

Similarly, Tables 7-10 on pages 24-25 show similar synergy against various bacterial and fungal organisms, when GV was combined with other basic reagents on the surfaces of medical devices.

Furthermore, as set forth in the Declaration of one of the inventors, Dr. Issam Raad (filed with the response to the Office Action dated January 11, 2006), additional evidence was provided demonstrating that the combination of a basic reagent and a dye has antiseptic ability as a mouthwash, coating of a glove, or coating of a catheter than is more than additive compared to either dye alone or basic reagent alone.

In addition, the second Declaration of Dr. Issam Raad ("the Second Declaration; Exhibit A of the response to the Office Action dated July 25, 2006), sets forth a summary of data from his laboratory that further demonstrates a high level of synergy of the combination of a basic reagent and a dye in antiseptic ability. Second Declaration, ¶8 and Exhibit 1 of Second Declaration.

Dr. Raad notes that the most serious forms of catheter related bloodstream infections are those caused by fungi, particularly *Candida albicans*. Second Declaration, ¶9. This is the infection with the highest mortality rate – around 40%. *Id.* His laboratory team found that gendine (GV and CHX) mixed in a specific molar ratio to coat catheters and devices provides unexpectedly superior synergy against *Candida albicans*. *Id.* The strain used in the studies summarized in Exhibit 1 was obtained from a patient who suffered from catheter-related fungemia/candidemia caused by *Candida albicans* (strain 009-3072). *Id.* In the first part of the study summarized in Exhibit 1, they calculated a minimal inhibitor concentration (MIC) and minimal fungicidal concentration (MBC) for each of the components, GV and CHX. The MIC/MBC was 0.5 microgram per mL for the GV and 16 microgram per mL for CHX. *Id.*

Dr. Raad's group then tested for synergy of the combination of CHX and GV over a range of 1:1 to 100:1, and obtained the results described on page 2 of Exhibit 1. Second Declaration, ¶10. Boxes that are shaded had a complete kill of the *Candida albicans* at the

respective concentrations of the components that are lower that the MIC and MBC of CHX alone and GV alone. *Id.* The best synergistic data was obtained at a ratio of CHX:GV of 1:1 and 10:1, with a plateauing effect at 25:1 and thereafter. *Id.* In other words, Dr. Raad's team found that there is synergy obtained at 50:1 and 100:1 but it is not appreciably different from 25:1. *Id.*

As noted by Dr. Raad, these results clearly establish that the claimed methods using a combination of a dye and basic reagent are surprisingly and unexpectedly superior compared to methods of disinfecting using dye alone or basic reagent alone. Second Declaration, ¶11.

Further, Dr. Raad's group has published a study (Exhibit 2 of response to Office Action dated August 21, 2007) that demonstrates that mouthwash compositions that have a ratio of dye:basic reagent of 10:1-66:1 demonstrated synergistic antimicrobial efficacy against free-floating and biofilm forms of *Candida albicans*. Second Declaration, ¶12.

The Examiner argues in the Office Action that the data in the specification and the information set forth in the Declarations of Dr. Raad are insufficient to demonstrate a synergistic effect of the claimed methods. In response to this assertion, Applicants have previously submitted the Declaration of Dr. Ray Hachem, (Exhibit B of response to Office Action dated August 21, 2007). Dr. Hachem is a skilled expert in infectious disease therapy and control. Hachem Declaration, ¶3. Dr. Hachem was asked whether the results set forth in the present patent application and the information set forth in the first and second Declarations of Dr. Raad demonstrate that the presently claimed invention demonstrates a synergistic antiseptic effect compared to dye alone or basic reagent alone. Hachem Declaration, ¶5. Dr. Hachem has cited to a definition of "synergy" from the 2007 instructions to authors from the journal entitled "Antimicrobial Agents and Chemotherapy." Hachem Declaration, ¶6. In paragraph 6 of his declaration, Dr. Hachem cites to specific sections of the patent application and evidence from the

declarations of Dr. Raad which demonstrate synergy. He concludes that the results set forth in the specification and the data cited in the Raad declarations clearly establishes that compositions that include a dye and basic reagent in the molar ratios set forth in the claims exhibit surprising and unexpected synergy for disinfecting and/or sterilizing surfaces or a fluid compared to dye alone or basic reagent alone. Hachem Declaration, ¶8.

As further evidence of the surprising and unexpected superior results of the claimed methods, Applicants herein submit the third declaration of Dr. Issam Raad (Exhibit B, "Third Declaration"). Dr. Raad is one of the inventors of the present application. He has attached as Exhibit 1 of his declaration a summary of results of studies which demonstrate the synergism between gentian violet and chlorhexidine, and between brilliant green and chlorhexidine. Third Declaration, ¶2. Table 1 and Table 2 demonstrate the results for a mouthwash solution which includes brilliant green and chlorhexidine, and Table 3 shows the results for silicone catheter segments coated with a composition that includes gentian violet and chlorhexidine. *Id.*

The results provided by Dr. Raad in Table 1 show lack of antiseptic activity produced by each of brilliant green and chlorhexidine when each was tested alone, in the concentrations shown in the table. Third Declaration, ¶3, citing to Exhibit 1. However when brilliant green and chlorhexidine were used together, in those same concentrations, the solution was able to completely inhibit the growth of *Klebsiella pneumoniae*, MRSA, and *Candida albicans*. *Id.* A solution that includes 0.004 mg/ml brilliant green and 0.006% chlorhexidine corresponds to a molar ratio of 22:1 brilliant green:chlorhexidine. A solution that includes 0.008 mg/ml brilliant green and 0.006% chlorhexidine corresponds to a molar ratio of 44:1 brilliant green:chlorhexidine. A solution that includes 0.008 mg/ml brilliant green and 0.012%

chlorhexidine corresponds to a molar ratio of 22:1 brilliant green:chlorhexidine. The results in Table 1 indicate that there is synergism between brilliant green and chlorhexidine. *Id.*

The results in Table 2 show lack of antiseptic activity produced by each of brilliant green and chlorhexidine when each was tested alone, in the concentrations shown in the Table. Third Declaration, ¶4, citing to Exhibit 1. However when brilliant green and chlorhexidine were used together, in those same concentrations, the solution was able to completely inhibit the growth of *Candida albicans*. *Id.* A solution that includes 0.008 mg/ml brilliant green and 0.012% chlorhexidine corresponds to a molar ratio of 22:1 brilliant green:chlorhexidine. A solution that includes 0.008 mg/ml brilliant green and 0.024% chlorhexidine corresponds to a molar ratio of 11:1 brilliant green:chlorhexidine. The results in Table 2 indicate that there is synergism between brilliant green and chlorhexidine. *Id.*

The results in Table 3 show that silicone catheter segments coated with a solution that includes gentian violet and chlorhexidine produced zones of inhibition, against Methicillin resistant *Staphylococcus aureus* (MRSA), while segments coated with either gentian violet alone or chlorhexidine alone failed to produce any zones of inhibition. Third Declaration, ¶5, citing to Exhibit 1. Zones of inhibition indicate absence of bacterial growth within that zone. The results in row 1 correspond to a molar ratio of 2.6:1 of chlorhexidine:gentian violet. The results in row 2 correspond to a molar ratio of 1.6:1 of chlorhexidine:gentian violet. The results in Table 3 indicate that there is synergism between gentian violet and chlorhexidine. *Id.*

This unexpected high level synergy whereby either agent alone has no effect (zones of inhibition of zero) and the combination results in sizable zones of inhibition has been demonstrated in various experiments presented in the patent application (see, *e.g.*, Tables 2, 3, 4,

6, 8 and 10). For example, neither gentian violet or chlorhexidine had any activity or zones of inhibition against *Pseudomonas aeruginosa* (PS4025) on the surfaces of endotracheal tubes, silicone catheters and silk sutures as shown in Tables 2, 3, 4 and 6, pages 8-9 of the patent application, whereas the combination (gendine) had large zones of inhibition. Similar results are shown on Tables 8 and 10 on page 10 for gentian violet with other basic agents, such as chloroxylenol or chlofoctol against other bacteria (*Alcaligenes faecalis*) or fungi (*C. parapsilosis*) that often cause serious infections in hospitalized patients. **Therefore, the fact that neither agent alone has any activity (zones of zero or confluent growth) and the combination results in large zones and total inhibition of growth reflects the non-obvious unexpected nature of this invention.**

Thus, to the extent that the Examiner might have set forth any *prima facie* case of obviousness, it has been successfully rebutted.

3. Rejections Based on Luthra and Perelin in View of Ibsen Are Overcome

Claims 97, 101, 102, 120, and 123 are rejected under 35 U.S.C. §103(a) as being unpatentable over Luthra and Perelin, and further in view of Ibsen *et al.* (U.S. Patent 4,204,978; "Ibsen"). The Examiner argues that while Luthra and Perelin teach or suggest the combination of chlorhexidine and gentian violet, and motivation to substitute gentian violet with brilliant green is provided by Ibsen. Applicants respectfully traverse.

Each of the cited claims includes brilliant green as the dye. For the reasons set forth above, the discussion of which is incorporated into this section, one of ordinary skill in the art would not be motivated to combine the teachings of Perelin with Luthra because Luthra, as discussed above, does not concern application of a chlorhexidine-containing solution to a medical device (as required by claim 69 and dependent claims). Further, nothing in either reference provides any teaching or suggestion for a method of sterilizing or disinfecting a fluid.

Neither reference appears to concern itself with sterilizing or disinfecting a fluid. Ibsen does not remedy the deficiency of Perelin and Luthra because it is only cited as teaching brilliant green.

Even if the Examiner argues that a *prima facie* case of obviousness has been made, which Applicants as discussed above assert is not the case, Applicants would still overcome any obviousness rejection because Applicants' methods are surprisingly and unexpectedly superior compared to methods using either dye alone or basic reagent alone. These results have been discussed in detail in the previous section.

In view of the foregoing, the rejection of claims 97, 101, 102, 120, and 123 under 35 U.S.C. §103(a) as being unpatentable over Luthra and Perelin, and further in view of Ibsen should be withdrawn.

4. Rejection Based on Perelin in view of Ibsen

Claim 98 is rejected under 35 U.S.C. §103(a) as being unpatentable over Perelin in view of Ibsen. The Examiner argues that the claim is unpatentable because the dental art typically uses swabs and/or gauze for dabbing and a dropper for dropping, and therefore the claimed invention is obvious. Applicants respectfully traverse.

Neither Perelin nor Ibsen teaches or suggests any method for disinfecting and/or sterilizing any of the items set forth in the claim. Perelin at most concerns methods for applying a chlorhexidine containing solution to a tooth. Ibsen concerns a composition for detecting cracks in a tooth. Neither references provides a suggestion or motivation to provide for a composition containing brilliant green and chlorhexidine for the purpose of sterilizing or disinfecting any of the items as set forth in the claim. Thus, the Examiner has failed to establish a prima facie case of obviousness.

Furthermore, for the reasons set forth above, even if the Examiner has established a prima facie case of obviousness, such a case would be rebutted by Applicants' evidence of surprising and unexpected superior results, as set forth above.

In view of the foregoing, it is respectfully requested that this rejection should be withdrawn.

5. Rejection Based on Perelin in View of Mantelle Is Moot

Claim 105 is rejected under 35 U.S.C. §103(a) as being unpatentable over Perelin as applied to claim 103 above, and further in view of Mantelle (U.S. patent 6,562,363). The Examiner argues that the claimed invention would be obvious to one of ordinary skill in the art because while Perelin does not disclose clofoctol, motivation to substitute clofoctol in the teachings of Perelin would be provided by Mantelle. Applicants respectfully traverse.

Without conceding that claim 105 as originally written would have been unpatentable in view of Perelin and Mantelle, Applicants note that claim 105 has been canceled without prejudice or disclaimer. Applicants specifically reserve the right to prosecute this claim in a divisional or continuation application. In view of the foregoing, the rejection of claim 105 under 35 U.S.C. §103(a) as being unpatentable over Perelin in view of Mantelle is moot.

6. Rejection Based on Perelin in View of Darouiche Is Overcome

Claim 106 is rejected under 35 U.S.C. §103(a) as being unpatentable over Perelin as applied to claim 103 above, and further in view of Darouiche (U.S. Patent 5,853,745). Applicants respectfully traverse.

Without conceding that claim 106 as originally written would have been unpatentable in view of Perelin and Darouiche, Applicants note that claim 106 has been canceled without prejudice or disclaimer. Applicants specifically reserve the right to prosecute this claim in a

divisional or continuation application. In view of the foregoing, the rejection of claim 106 under 35 U.S.C. §103(a) as being unpatentable over Perelin in view of Darouiche is moot.

7. Rejection Based on Perelin in View of Curtis Is Overcome

Claim 107 is rejected under 35 U.S.C. §103(a) as being unpatentable over Perelin as applied to claim 103 above, and further in view of Curtis *et al.* (U.S. Patent 5,209,251; "Curtis"). Applicants respectfully traverse.

Without conceding that claim 107 as originally written would have been unpatentable in view of Perelin and Curtis, Applicants note that claim 107 has been canceled without prejudice or disclaimer. Applicants specifically reserve the right to prosecute this claim in a divisional or continuation application. In view of the foregoing, the rejection of claim 107 under 35 U.S.C. §103(a) as being unpatentable over Perelin in view of Curtis is moot.

8. Rejection Based on Perelin in View of Ibsen Is Overcome

Claim 108 is rejected under 35 U.S.C. §103(a) as being unpatentable over Perelin as applied to claim 103 above, and further in view of Ibsen. The Examiner argues that while Perelin fails to disclose the use of brilliant green as the dye, Ibsen provides motivation to substitute the gentian violet of Perelin with the brilliant green of Ibsen. Applicants respectfully traverse.

The Examiner has failed to set forth a *prima facie* case of obviousness because she has not set forth that Perelin in view of Ibsen teaches or suggests each limitation of the claimed invention. As discussed above, independent claim 103 (from which claim 108 depends) has been amended to recite that the surface which is being disinfected and/or sterilized is a mucosal surface. For the reasons set forth above, Perelin does not teach a method for disinfecting and/or sterilizing a mucosal surface. Rather, it appears to pertain to application of its composition to a

tooth surface (*e.g.*, dental root or dentinal tubule). Thus, even if one of ordinary skill in the art substituted gentian violet in Perelin with brilliant green, the claimed invention would still not be practiced because the surface taught by Perelin is not a mucosal surface.

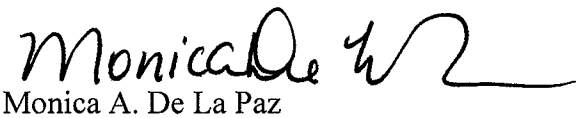
Further, even if the Examiner had set forth a *prima facie* case of obviousness, which Applicants assert is not the case, such a *prima facie* case would be successfully rebutted by Applicants' evidence of surprising and unexpected superior results of the claimed methods, as set forth above. The Examiner is particularly directed to the evidence set forth in the third Declaration of Dr. Raad, discussed above.

In view of the foregoing, claim 108 is not unpatentable under 35 U.S.C. §103(a) based on the combination of Perelin in view of Ibsen. Therefore, Applicants respectfully request that this rejection should be withdrawn.

E. Conclusion

In view of the foregoing, it is respectfully submitted that each of the pending claims is in condition for allowance. The Examiner is invited to contact the undersigned attorney at (512) 536-5639 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,



Monica A. De La Paz
Reg. No. 54,662
Attorney for Applicants

FULBRIGHT & JAWORSKI L.L.P.
600 Congress Avenue, Suite 2400
Austin, Texas 78701
512.474.5201 (telephone)
512.536.4598 (fax)

Date June 11, 2008

EXHIBIT A

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Stedman's
Medical Dictionary

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dental lamina *n.* A band of ectodermal cells growing from the epithelium of the embryonic jaws into the underlying mesenchyme and giving rise to the primordia of the enamel organs of the teeth.
dental orthopedics *n.* Orthodontics.
dental plaque *n.* A film of mucus and bacteria on a tooth surface. Also called *bacterial plaque.*
dental plate *n.* See **denture** (sense 1).
dental polyp *n.* Hyperplastic pulpal tissue growing out of a decayed tooth with wide pulpal exposure.
dental pulp *n.* The soft tissue forming the inner structure of a tooth and containing nerves and blood vessels. Also called *tooth pulp.*
dental ridge *n.* The prominent border of a cusp or margin of a tooth.
dental sac *n.* The envelope of connective tissue surrounding a developing tooth.
dental surgeon *n.* A general practitioner of dentistry having a D.D.S. or D.M.D. degree.
dental syringe *n.* A breech-loading syringe fitted with a sealed cartridge containing anesthetic solution.
dental technician *n.* A person who makes dental appliances and restorative devices, such as bridges or dentures, to the specifications of a dentist.
den•tate (dĕn′tāt′) *adj.* Edged with toothlike projections; toothed.
dentate gyrus *n.* One of the two interlocking gyri composing the hippocampus.
dentate nucleus of cerebellum *n.* The most lateral and largest of the deep cerebellar nuclei, receiving axons of the Purkinje cells of the neocerebellum, and serving as a source of fibers composing the superior cerebellar peduncle.
dentate suture *n.* See **serrate suture.**
denti– or **dent–** or **dento–** *pref.* 1. Tooth: *dentalgia.* 2. Dental: *dentilabial.*
den•ti•cle (dĕn′tĭ-kəl) *n.* 1. See **pulp stone.** 2. A small tooth or toothlike projection.
den•ti•frice (dĕn′tə-frĭs′) *n.* A substance, such as a paste or powder, for cleaning the teeth.
den•tig•er•ous (dĕn-tĭj′ər-əs) *adj.* Having or furnished with teeth.
dentigerous cyst *n.* A cyst arising in the odontogenic epithelium after the crown of a developing tooth has been formed.
den•ti•la•bi•al (dĕn′tə-lā′bē-əl) *adj.* Relating to the teeth and lips.
den•ti•lin•gual (dĕn′tə-lĭng′gwəl) *adj.* Relating to the teeth and tongue.
den•tin (dĕn′tĭn) or **den•tine** (-tēn′) *n.* The main, calcareous part of a tooth, beneath the enamel and surrounding the pulp chamber and root canals.
den•tin•al (dĕn′tə-nəl, dĕn-tē′-) *adj.* Of or relating to dentin.
dentinal canal *n.* Any of the minute, wavy, branching tubes in the dentin that contain dentinal fibers and extend radially from the pulp to the dentoenamel junction. Also called *dentinal tubule.*
dentinal dysplasia *n.* See **dentinogenesis imperfecta.**
den•ti•nal•gi•a (dĕn′tə-năl′jē-ə, -jə) *n.* Pain in the dentin.
dentinal lamina cyst *n.* A small keratin-filled cyst that is derived from remnants of the dental lamina and usually appears in groups on the alveolar ridge of newborns.
dentinal papilla *n.* A projection of the mesenchymal tissue of the developing jaw into the cup of the enamel organ; its outer layer becomes odontoblasts that form the dentin of the tooth.
dentinal sheath *n.* A relatively acid-resistant layer of tissue that forms the walls of the dentinal tubules.
dentinal tubule *n.* See **dentinal canal.**
dentin dysplasia *n.* A hereditary disorder of both the primary and permanent teeth characterized by short roots, obliteration of the pulp chambers and canals, and mobility and premature loss.
den•ti•no•ce•men•tal (dĕn′tə-nō-sĭ-mĕn′tl) *adj.* Of, relating to, or characteristic of the dentin and cementum of teeth.
dentinocemental junction *n.* See **cementodentinal junction.**
den•ti•no•e•nam•el (dĕn′tə-nō-ĭ-năm′əl) *adj.* Relating to or characteristic of the dentin and the enamel of teeth.
dentinoenamel junction *n.* The surface at which the enamel and the dentin of the crown of a tooth are joined. Also called *amelodental junction, amelodentinal junction.*
den•ti•no•gen•e•sis (dĕn′tə-nō-jĕn′ĭ-sĭs) *n.* The formation of dentin.
dentinogenesis im•per•fec•ta (ĭm′pər-fĕk′tə) *n.* A hereditary defect of dentin formation characterized by a translucent or opalescent color of the teeth, easy fracturing of the enamel, wearing of occlusal surfaces, and staining of exposed dentin. Also called *dentinal dysplasia, hereditary opalescent dentin.*
den•ti•noid (dĕn′tə-noid′) *adj.* Resembling dentin. —*n.* See **dentinoma.**
den•ti•no•ma (dĕn′tə-nō′mə) *n.* A tumor containing dentin, occuring in the tissues that form teeth. Also called *dentinoid.*
den•ti•num (dĕn′tə-nəm, dĕn-tī′nəm) *n.* Dentin.
den•tip•a•rous (dĕn-tĭp′ə-rəs) *adj.* Bearing teeth.
den•tist (dĕn′tĭst) *n.* A person who is trained and licensed to practice dentistry.
den•tist•ry (dĕn′tĭ-strē) *n.* The science concerned with the prevention, diagnosis, and treatment of diseases of the teeth, gums, and related structures of the mouth and including the repair or replacement of defective teeth.
den•ti•tion (dĕn-tĭsh′ən) *n.* 1. The natural teeth, considered collectively, in the dental arch. 2. The type, number, and arrangement of a set of teeth. 3. The process of growing new teeth; teething.
dento– *pref.* Variant of **denti–.**
den•to•al•ve•o•lar (dĕn′tō-ăl-vē′ə-lər) *adj.* 1. Relating to a tooth and the part of the alveolar bone that immediately surrounds it. 2. Relating to the functional unity of the teeth and the alveolar bone.
dentoalveolar abscess *n.* An abscess confined to the dentoalveolar process enclosing a tooth root.

den•tu•lous (dĕn′chə-ləs) *adj.* I
den•ture (dĕn′chər) *n.* 1. A pa artificial teeth for either th Also called *dental plate.* 2. d of removable artificial teeth
denture base *n.* 1. The part of the oral mucus membrane attached. 2. The part of a c ture that rests on the dent which teeth are attached.
denture foundation *n.* The pc ture that can be used to sup
de•nu•cle•at•ed (dē-nōō′klē- prived of a nucleus.
de•nu•da•tion (dē′nōō-dā′sha The removal of a covering c
de•nude (dĭ-nōōd′, -nyōōd′) **-nudes.** To divest of a cover
de•o•dor•ant (dē-ō′dər-ənt) *n.* suppresses, or neutralizes od applied to the skin to mask pable of masking, suppressir
de•o•dor•ize (dē-ō′də-rīz′) *v.* mask or neutralize the odo
tion (-dər-ī-zā′shən) *n.*
de•o•dor•iz•er (dē-ō′də-rī′z masks or neutralizes odors.
de•os•si•fi•ca•tion (dē-ŏs′ə-fĭ- removal of the mineral con
deoxy– or **desoxy–** *pref.* Losir oxygen: *deoxyadenosine.*
de•ox•y•a•den•o•sine (dē-ŏk of the four principal nucleo of adenine and deoxyribose
de•ox•y•ad•e•nyl•ic acid (d nucleotide formed in the h called *adenine deoxyribonu*
de•ox•y•cho•lic acid (dē-ŏk′ used as a choleretic and dig sis of adrenocortical hormo
de•ox•y•cor•ti•cos•ter•one rōn′) *n.* A steroid hormon or by the adrenal cortex a insufficiency. Also called 2
de•ox•y•cy•ti•dine (dē-ŏk′sē- principal nucleosides of DN and deoxyribose.
de•ox•y•cy•ti•dyl•ic acid (d nucleotide formed in the h
de•ox•y•gen•a•tion (dē-ŏk process of removing dissol such as water.
de•ox•y•gua•no•sine (dē-ŏk One of the principal nuclec of guanine and deoxyribos
de•ox•y•gua•nyl•ic acid (d nucleotide formed in the called *guanine deoxyribon*
de•ox•y•ri•bo•nu•cle•ase (d -āz′, -nyōō′-) *n.* DNase.

of deep muscular manipulation and massage for the relief of bodily and emotional tension.

roll•er bandage (rō′lər) *n.* A strip of material, of variable width, rolled into a compact cylinder to facilitate application.

Rom•berg's sign (rŏm′bərgz) *n.* A sign indicating loss of proprioceptive control in which increased unsteadiness occurs when standing with the eyes closed compared with standing with the eyes open. Also called *rombergism.*

ron•geur (rôn-zhœr′, rôN-) *n.* A heavy-duty forceps for removing small pieces of bone.

rönt•gen (rĕnt′gən, -jən, rŭnt′-) *n.* Variant of **roentgen.**

roof (rōōf, rŏŏf) *n.* The upper surface of an anatomical structure, especially one having a vaulted inner structure.

roof of fourth ventricle *n.* The roof of the fourth ventricle formed by the inferior and the superior medullary vela.

roof of tympanum *n.* The roof of the middle ear, formed by the thinned anterior surface of the petrous portion of the temporal bone.

roof plate or **roofplate** *n.* The thin layer of the embryonic neural tube that connects the lateral plates dorsally.

root (rōōt, rŏŏt) *n.* **1.** The embedded part of an organ or structure, such as a hair, tooth, or nerve, serving as a base or support. **2.** A primary source; an origin; radix.

root amputation *n.* Surgical removal of one or more roots of a multirooted tooth. Also called *radectomy.*

root canal *n.* **1.** The chamber of the dental pulp lying within the root portion of a tooth. Also called *pulp canal.* **2.** A treatment in which diseased tissue from this part of the tooth is removed and the resulting cavity is filled with an inert material.

root caries index *n.* The ratio of the number of teeth with carious lesions of the root and restorations of the root to the number of teeth with exposed root surfaces.

root filament *n.* Any of the small individual fiber fascicles into which the roots of each spinal nerve and several of the cranial nerves divide in a fanlike fashion before entering or leaving the spinal cord or brainstem. Also called *nerve rootlet.*

root•ing reflex (rōō′tĭng, rōōt′ĭng) *n.* A reflex in infants in which rubbing or scratching about the mouth causes the infant to turn its head toward the stimulus.

root of lung *n.* All the structures entering or leaving the lung at the hilum.

root of nail *n.* The proximal end of the nail, concealed under a fold of skin.

root of penis *n.* The proximal attached part of the penis, including the two crura and the bulb.

root of tongue *n.* The rear attached portion of the tongue.

root of tooth *n.* The part of a tooth below the neck of the tooth, covered by cementum rather than enamel and attached by the periodontal ligament to the alveolar bone.

root resection *n.* See **apicoectomy.**

root sheath *n.* Either of two epidermic layers of the hair follicle.

Ror•schach (rôr′shäk′, -shäkʜ′), **Hermann.** 1884–1922. Swiss psychiatrist. His inkblot test, introduced in 1921, has become a standard clinical diagnostic tool in psychiatry.

ro•sa•ce•a (rō-zā′shē-ə) *n.* A chronic dermatitis of the face, especially of the nose and cheeks, characterized by a red or rosy coloration with deep-seated papules and pustules and caused by dilation of capillaries. Also called *acne erythematosa, acne rosacea.*

ros•an•i•line or **ros•an•i•lin** (rō-zăn′ə-lĭn) *n.* A brownish-red crystalline organic compound derived from aniline and used in the manufacture of dyes and in Schiff's reagent.

ro•sa•ry (rō′zə-rē) *n.* An arrangement or structure that is beadlike in appearance.

rose ben•gal (rōz′ bĕn-gôl′, bĕng-, bĕn′gəl, bĕng′-) *n.* A bluish-red dye used as a stain for bacteria, as a stain in the diagnosis of keratitis sicca, and in tests of liver function.

rose fever *n.* A spring or early summer hay fever. Also called *rose cold.*

ro•se•o•la (rō-zē′ə-lə, rō′zē-ō′lə) *n.* A rose-colored skin rash, sometimes occurring with diseases such as measles, syphilis, or scarlet fever.

roseola in•fan•tum (ĭn-făn′təm) *n.* See **exanthema subitum.**

Rose's position (rōz′zĭz) *n.* A supine position with the head over the end of a table, used for operations within the mouth or the fauces.

rose spots *pl.n.* The characteristic rose-colored spots of typhoid fever.

ro•sette (rō-zĕt′) *n.* **1.** The segmented or mature phase of *Plasmodium malariae.* **2.** A grouping of cells characteristic of neoplasms of neuroblastic or neuroectodermal origin, in which a number of nuclei form a ring from which neurofibrils extend to interlace in the center.

ros•in (rŏz′ĭn) *n.* A translucent yellowish to dark brown resin derived from the stumps or sap of various pine trees and used as an adhesive in plasters and as a stimulant in ointments.

Ross (rôs), Sir **Ronald.** 1857–1932. British physician. He won a 1902 Nobel Prize for proving that malaria is transmitted to humans by the bite of the mosquito.

Ros•so•li•mo's reflex (rŏs′ə-lē′mōz) *n.* **1.** Flexion of the toes in response to flicking the tips of the underside of the toes, indicative of lesions of the pyramidal tracts. **2.** Flexion of the fingers in response to tapping the tips of the fingers on their volar surfaces. Also called *Rossolimo's sign.*

Ross River virus *n.* A mosquito-borne alphavirus that causes epidemic polyarthritis.

ros•tel•lum (rŏ-stĕl′əm) *n., pl.* **ros•tel•la** (rŏ-stĕl′ə). A small beaklike part, such as the hooked projection on the head of a tapeworm.

EXHIBIT B

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Issam RAAD, Hend A. HANNA, and Nabeel NABULSI	Group Art Unit: 1744
	Examiner: McKane, Elizabeth L.
Serial No.: 10/044,842	Atty. Dkt. No.: UTSC:669US
Filed: January 11, 2002	
For: NOVEL ANTISEPTIC DERIVATIVES WITH BROAD SPECTRUM ANTIMICROBIAL ACTIVITY FOR THE IMPREGNATION OF SURFACES	Confirmation No. 7921

THIRD DECLARATION OF DR. ISSAM RAAD

I, Dr. Issam Raad, hereby declare as follows:

1. I am one of the inventors of the above-referenced patent application. I am a citizen of the U.S., currently residing at 4207 Clearwater Ct., Missouri City, TX, 77459.

2. I have attached as Exhibit 1 of this declaration a summary of studies which have been conducted which demonstrate the synergism between gentian violet and chlorhexidine, and between brilliant green and chlorhexidine. Table 1 and Table 2 demonstrate the results for a mouthwash solution which includes brilliant green and chlorhexidine, and Table 3 shows the results for silicone catheter segments coated with a composition that includes gentian violet and chlorhexidine.

3. The results in Table 1 show lack of antiseptic activity produced by each of brilliant green and chlorhexidine when each was tested alone, in the concentrations shown in the table. However when brilliant green and chlorhexidine were used together, in those same concentrations, the solution was able to completely inhibit the growth of *Klebsiella pneumoniae*, MRSA, and *Candida albicans*. A solution that includes 0.004 mg/ml brilliant green and 0.006% chlorhexidine corresponds to a molar ratio of 22:1 brilliant green:chlorhexidine. A solution that includes 0.008 mg/ml brilliant green and 0.006% chlorhexidine corresponds to a molar ratio of 44:1 brilliant green:chlorhexidine. A solution that includes 0.008 mg/ml brilliant green and 0.012% chlorhexidine corresponds to a molar ratio of 22:1 brilliant green:chlorhexidine. The results in Table 1 indicate that there is unexpected high level synergism between brilliant green and chlorhexidine.

4. The results in Table 2 show lack of antiseptic activity produced by each of brilliant green and chlorhexidine when each was tested alone, in the concentrations shown in the Table. However when brilliant green and chlorhexidine were used together, in those same concentrations, the solution was able to completely inhibit the growth of *Candida albicans*. A solution that includes 0.008 mg/ml brilliant green and 0.012% chlorhexidine corresponds to a molar ratio of 22:1 brilliant green:chlorhexidine. A solution that includes 0.008 mg/ml brilliant green and 0.024% chlorhexidine corresponds to a molar ratio of 11:1 brilliant green:chlorhexidine. The results in Table 2 indicate that there is unexpected high level synergism between brilliant green and chlorhexidine.

5. The results in Table 3 show that silicone catheter segments coated with a solution that includes gentian violet and chlorhexidine produced zones of inhibition, against methicillin resistant *Staphylococcus aureus* (MRSA), while segments coated with either gentian violet alone or chlorhexidine alone failed to produce any zones of inhibition. Zones of inhibition indicate absence of bacterial growth within that zone. The results in row 1 correspond to a molar ratio of 2.6:1 of chlorhexidine:gentian violet. The results in row 2 correspond to a molar ratio of 1.6:1 of chlorhexidine:gentian violet. The results in Table 3 indicate that there is unexpected high level synergism between gentian violet and chlorhexidine. This unexpected high level synergy whereby either agent alone has no effect (zones of inhibition of zero) and the combination results in sizable zones of inhibition has been demonstrated in various experiments presented in the patent application (Tables 2, 3, 4, 6, 8 and 10). For example, neither gentian violet or chlorhexidine had any activity or zones of inhibition against *Pseudomonas aeruginosa* (PS4025) on the surfaces of endotracheal tubes, silicone catheters and silk sutures as shown in Tables 2, 3, 4 and 6, pages 8-9 of the patent application, whereas the combination (gendine) had large zones of inhibition. Similar results are shown on Tables 8 and 10 on page 10 for gentian violet with other basic agents, such as chloroxylenol or chlofoctol against other bacteria (*Alcaligenes faecalis*) or fungi (*C. parapsilosis*) that often cause serious infections in hospitalized patients. **Therefore, the fact that neither agent alone has any activity (zones of zero or confluent growth) and the combination results in large zones and total inhibition of growth reflects the non-obvious unexpected nature of this invention.**

6. I hereby declare that all statements made by my own knowledge are true and all statements made on information and belief are believed to be true and further that statements

were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment under § 100 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issued thereon.

Date 5/16/08

I Raad

Dr. Issam Raad

EXHIBIT 1

Table 1: Synergism of chlorhexidine and brilliant green in solutions towards three free-floating microorganisms. The last solution (0.008/0.012%) was tested against MRSA only, expressed as CFU/disk.

	Klebsiella pneumoniae* CFU	**MRSA CFU**	***Candida albicans* CFU**
Saline (Control)	>5000	>5000	>5000
0.004 mg/mL brilliant green	>5000	>5000	>5000
0.008 mg/mL brilliant green	>5000	>5000	>5000
0.006 % chlorhexidine	>5000	>5000	400
0.004 mg/ml brilliant green + 0.006% chlorhexidine	0	0	0
0.008 mg/ml brilliant green + 0.006% chlorhexidine	0	0	0
0.012 % chlorhexidine	0	>5000	100
0.008 mg/ml brilliant green + 0.012% chlorhexidine	0	0	0

*CFU = colony forming units; MRSA = methicillin-resistant *Staphylococcus aureus*

Results in table 1show lack of antiseptic activity produced by each of brilliant green and chlorhexidine when each was tested alone, in the concentrations shown in the table. However when brilliant green and chlorhexidine were used together, in those same concentrations, the solution was able to completely inhibit the growth of *Klebsiella pneumoniae*, MRSA, and *Candida albicans*. These results suggest synergism between brilliant green and CHX.

Table 2: Synergism of chlorhexidine and brilliant green in solutions against *Candida albicans* biofilm form, expressed as CFU*/disk.

	Candida albicans
Solution	**Mean CFU/disk ± SE**
Saline (control)	>5000, >5000, >5000
0.008 brilliant green	>5000, >5000, >5000
0.012 chlorhexidine	>5000, >5000, >5000
0.024 chlorhexidine	>5000, >5000, >5000
0.008 brilliant green + 0.012 chlorhexidine	0, 0, 0
0.008 brilliant green + 0.024 chlorhexidine	0, 0, 0

*CFU = colony forming units; MRSA = methicillin-resistant *Staphylococcus aureus*.

Results in table 2 show lack of antiseptic activity produced by each of brilliant green and chlorhexidine when each was tested alone, in the concentrations shown in the table. However when brilliant green and chlorhexidine were used together, in those same concentrations, the solution was able to completely inhibit the growth of *Candida albicans*. These results suggest synergism between brilliant green and CHX.

Table 3: Synergism of gentian violet (GV) and chlorhexidine (CHX). Antiseptic silicone catheter segments

	Sample ID* Of catheter	GV/CHX**	Baseline*** (7-8-03)	1 Week (7-15-03)	2 Week (7-22-03)	3 Week (7-29-03)	4 Week (8-6-03)	5 Week (8-13-03)	6 Week (8-20-03)
1	Si030620-com1	0.25/1.0g	12 : 12	10 : 9	9 : 9	9 : 9	8 : 8	0 : 0	0 : 0
2	Si030620-com2	0.416/1.0g	14 : 14	11 : 11	9 : 9	8 : 8	10 : 10	7 : 7	8 : 8
	-								
3	Si030620-sep1	None/1.0g	0 : 0	0 : 0					
4	Si030620-sep2	0.25g/None	0 : 0	0 : 0					
5	Si030620-sep3	0.416g/None	0 : 0	0 : 0					

*Silicone catheter segments coated with the corresponding concentrations of GV/CHX
Row 1, silicone segments were coated with combination of GV (0.25gm) and CHX (1.0gm)
Row 2, silicone segments were coated with combination of GV (0.41gm) and CHX (1.0gm)
Row 3, silicone segments were coated only with CHX
Row 4, silicone segments were coated only with GV
Row 5, silicone segments were coated only with GV
***Zones of inhibition, measured in millimeters, around tested catheter segments at baseline and at weekly intervals up to 6 weeks. Catheter segments were soaked in serum that was changed weekly, after the removal of the segment to be tested that particular week.

Results in table 3 show that silicone catheter segments coated with the GV/CHX produced zones of inhibition, against Methicillin resistant *Staphylococcus aureus* (MRSA), while segments coated with either GV alone or CHX alone failed to produce any zones of inhibition. Zones of inhibition indicate absence of bacterial growth within that zone. These results suggest synergism between GV and CHX.